

# Southern and Northern blot fixing by microwave oven

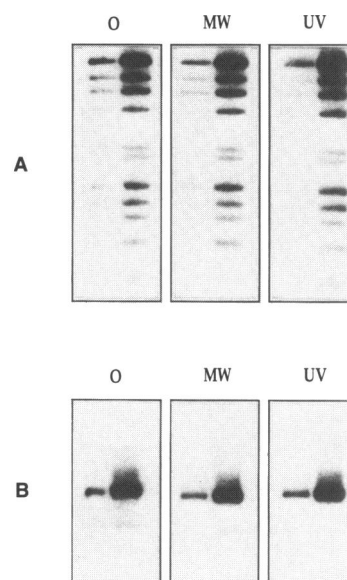
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Southern and Northern blot techniques are powerful procedures for studying gene structure and function. These methods involve the separation of nucleic acids according to size on an agarose or polyacrylamide gel and subsequent transfer of the nucleic acid to suitable nitrocellulose or nylon-based membranes. DNA or RNA molecules bound to the membrane are then fixed and hybridized with a radioactive probe, specific for the gene under study. The fixing step is particularly important for quantitatively reproducible results, especially if membranes are to be rehybridized several times. The traditional fixing procedure involved baking the membrane for 2 h at 80°C (1). More recently, it has been shown that efficient cross-linking of nucleic acids to the membrane could be obtained by 30 s to 3 min exposure of UV irradiation (2). Earlier studies described the use of a microwave oven to fix bacterial colonies to nylon membranes (3) or to fix DNA within paraffin tissue blocks (4). We demonstrate here that a 2 min treatment of Southern or Northern blots in a microwave oven efficiently fixes nucleic acids to nylon membranes which can withstand repeated reuse in hybridization reactions.

Serial dilutions of *Hind*III digested  $\lambda$  DNA and *Hae*III digested  $\phi$ X174 DNA were separated by electrophoresis through a 1% agarose gel in TAE buffer. Messenger RNA was extracted from rat pheocromocytoma (PC12) cells with the Quick Prep Micro mRNA purification kit (Pharmacia) and electrophoresed through a 1.3% agarose-formaldehyde gel (5). DNA or RNA were blotted on a nylon-based membrane (Hybond N, Amersham) in 10×SSC by vacuum blotting. The membrane was divided into three parts, layered on a piece of paper Whatman 3 MM and, still moist, DNA and RNA were fixed as follows: by baking for 2 h at 80°C by UV irradiation (254 nm for 1 min and 45 s, 1.5 J/cm<sup>2</sup>); by 2 min and 30 s treatment in a microwave oven at full setting (750–900 W). The probes were prepared by random priming with [ $\alpha$ -<sup>32</sup>P]dCTP using a commercial kit (Ready-To-Go, Pharmacia Biotech). Southern blots were hybridized with  $\lambda$ /*Hind*III and/or  $\phi$ X174/*Hae*III digested DNAs, whereas a cDNA clone of hsc73 (heat shock constitutive of 73 kD protein) rat gene was used for Northern blot hybridization. Pre-hybridization and hybridization of both Southern and Northern blots were performed according to conventional methods using a hybridization solution containing 0.5 M NaCl, 0.1 M NaPO<sub>4</sub> pH 6.8, 10 mM EDTA, 1% SDS, 1% BSA, 100 µg/ml sonicated salmon sperm DNA with 50% formamide (Northern blot) or without (Southern blot). Prior to re-hybridization, the membrane was washed at 95°C for a few minutes in 0.1×SSC, 1% SDS. The optimal microwave fixation time of 2 min and 30 s resulted from a time course treatment



**Figure 1.** Hybridization to DNA and RNA fixed to a nylon membrane by different methods, the figure shows the result after eight cycles of probe removal by boiling and rehybridization for Southern and three cycles for Northern blots. Aliquots of 0.5 and 5 ng of either *Hind*III digested  $\lambda$  and *Hae*III digested  $\phi$ X174 DNAs for Southern blot and 0.2 and 1 µg of PC12 mRNA for Northern blot were transferred to Hybond N (Amersham) membrane after electrophoresis. Subsequently the membrane was cut in three parts and the DNAs and RNA were fixed by: O) baking for 2 h at 80°C, UV) UV irradiation (254 nm for 1 min and 45 s, 1.5 J/cm<sup>2</sup>); MW) 2 min in a microwave oven at full setting (850 W). (A) Shows autoradiography of the three membranes hybridized together with both radioactive  $\lambda$  *Hind*III and  $\phi$ X174 DNAs. (B) Shows autoradiography of Northern blots hybridized together with a cDNA clone of rat hsc73.

starting from 0 to 5 min. No over treatment effect was observed by microwave fixing.

As Figure 1 shows, the hybridization signal obtained using microwave oven fixed membrane, is comparable to that obtained with the other two methods. In all experiments the microwave oven treatment produced excellent results for both Southern and Northern blots. All three fixing procedures always produced a signal at least double compared to no treatment. In some experiments, both Southern and Northern blots were reprobed up to nine times. No appreciable loss of the sensitivity was observed

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in all the three methods. Beside Hybond N, N<sup>+</sup> and Nfp (Amersham), we tested other brands of membranes such as GeneScreen (NEN Dupont), Zetaprobe (Bio-Rad) as well as Nitrocellulose (Hybond C, Amersham). Microwave fixation resulted suitable for all tested membranes. No appreciable differences were observed among the three methods of fixation for all nylon-based membranes. Only for nitrocellulose membrane microwave fixation produced a decreased signal intensity compared to oven baking treatment. These results indicate that microwave fixing is comparable to conventional or UV fixation methods, but has the advantage that it is at least as rapid as UV treatment and does not require the specialized and expensive equipment needed for UV cross-linking.

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